

APPLICANTS: Stanley T. Crooke
SERIAL NO: 09/479,783

DOCKET NO: ISIS-4313

AMENDMENTS TO THE SPECIFICATION:

Please replace the paragraph on page 1, lines 3-5, with the following:

CROSS REFERENCED TO RELATED APPLICATIONS

This application is a divisional of U.S. Serial Number 08/870,608, filed June 6, 1997, which is a continuation in part of U.S. Serial Number 08/659,440 filed June 6, 1996.

Please replace the paragraph beginning on page 15, line 30 through page 16, line 8, with the following:

Figure 7 shows analysis of products of digestion of dsRNase substrates by native polyacrylamide gel electrophoresis. Antisense and sense oligonucleotides were preannealed and incubated with cellular extracts and purified dsRNases as ~~described~~ described herein. Lane 1, untreated "sense" strand RNA; lane 2, "sense" strand RNA treated with 0.02 units RNase V1; remaining lanes: dsRNase substrates treated with 0.02 (lane 3) and 0.002 (lane 4) units of RNase V1, with unpurified nuclear extract for 0 minutes (lane 5) or 240 minutes (lane 6), with unpurified nuclear extract for 240 minutes without Mg^{++} (lane 7), with unpurified cytosolic extract for 240 minutes (lane 8), with ion exchange purified cytosolic extract for 240 minutes in the presence (lane 9) or absence (lane 10) of Mg^{++} , and with ion exchange/gel filtration purified cytosolic extract for 240 minutes in the presence (lane 9) or absence (lane 10) of Mg^{++} .

Please replace the paragraph on page 33, lines 5-26, with the following:

2'-O-Methyl nucleoside amidites and 2'-OH (blocked as 2'-*t*-butyldimethylsilyl derivative) nucleoside amidites are available from Glen Research, Sterling, VA. Other 2'-O-alkyl ~~substituted~~ substituted nucleoside amidites are prepared as is described in U.S. Patents 5,506,351, 5,466,786 or 5,514,786, herein incorporated by reference. Cyclobutyl sugar surrogate compounds are prepared as is described in U.S. Patent 5,359,044, herein incorporated by reference. Pyrrolidine sugar surrogate are prepared as is described in U.S. Patent 5,519,134, herein incorporated by reference. Morpholino sugar surrogates are prepared as is described in U.S. Patents 5,142,047 and 5,235,033, herein incorporated by reference, and other related patent disclosures. N-2 ~~substituted~~ substituted purine nucleoside amidites are prepared as is described in U.S. Patent 5,459,255, herein incorporated by reference. 3-Deaza purine nucleoside amidites are prepared as is described in U.S. Patent

APPLICANTS: Stanley T. Crooke
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DOCKET NO: ISIS-4313

5,457,191, herein incorporated by reference. 5,6-Substituted pyrimidine nucleoside amidites are prepared as is ~~described~~ described in U.S. Patent 5,614,617 herein incorporated by reference. 5-Propynyl pyrimidine nucleoside amidites are prepared as is described in U.S. Patent 5,484,908, herein incorporated by reference.

Please replace the paragraph on page 84, lines 17-34, with the following:

Duplexes used in the cell free T24 extract experiments were annealed as described above with the exception that after formation of the duplex, the reaction was treated with 1 μ l of a mixture RNase T and A (Ambion RPAII kit, Austin, TX) and incubated for 15 min at 37°C, and then gel purified from a nondenaturing 12% polyacrylamide gel. T24 cell nuclear and cytosolic fractions were isolated as described previously (Szyf, M., Bozovic, V., and Tanigawa, G., *J. Biol. Chem.*, 1991, 266, 10027-10030). Annealed duplexes (10 μ l) were incubated with 3 μ g of the T24 cytosolic extract at 37°C. The reaction was terminated by phenol/chloroform extraction and ethanol precipitated with the addition of 10 μ g of tRNA as a carrier. Pellets were resuspended in 10 μ l of denaturing loading dye, products were resolved on 12 % denaturing acrylamide gels as described above. 32 P-labeled 17-base RNA was hydrolysed by heating to 95°C for 10 ~~minutes~~ minutes in the presence of 50 mM NaCO₃, pH=9.0 to generate a molecular weight ladder.

Please replace the paragraph on page 92, lines 2-8, with the following:

Purification and characterization of double-stranded ribonucleases from ~~mamallian~~ mammalian tissues

In order to determine if ~~mamallian~~ mammalian cells, other than cultured cell lines, contain double-strand RNase activity, and to provide a source from which such ribonucleases might be purified, the following efforts were undertaken to identify and purify dsRNases from rat liver homogenates.

Please replace Table 1 on page 93 with the following Table:

TABLE 1

Artificial Substrates for ~~Mamallian~~ Mammalian dsRNases^{*}

Ha-ras TARGETED SENSE/ANTISENSE OLIGONUCLEOTIDES	
SEQ ID NO:1	5'-GGG CGC CGU CGG UGU GG-3'

APPLICANTS: Stanley T. Crooke
SERIAL NO: 09/479,783

DOCKET NO: ISIS-4313

SEQ ID NO:2	3'-CCC GCG GCA GCC ACA CC-5'
C-raf TARGETED SENSE/ANTISENSE OLIGONUCLEOTIDES	
SEQ ID NO:3	5'-CGG AAU GUG ACC GCC UCC CG-5'
SEQ ID NO:4	3'-GGC UUA CAC UGG CGG AGG GC-3'
	<u>5'-CCG AAU GUG ACC GCC UCC CG-3'</u>
	<u>3'-GGC UUA CAC UGG CGG AGG GC-5'</u>
SEQ ID NO:5	5'-UCA AUG GAG CAC AUA CAG GG-3'
SEQ ID NO:6	3'-AGU UAC CUC GUG UAU GUC CC-5'
SEQ ID NO:7	5'-AAU GCA UGU CAC AGG CGG GA-3'
SEQ ID NO:8	3'-UUA CGU ACA GUG UCC GCC CU-5'

Please replace Table 2 on page 96 with the following Table:

TABLE 2

Summary of Purification of dsRNases from Rat Liver Homogenates

Fraction	Protein (mg)	Total Activity (units [±])	Specific Activity (unit/mg)	Purifi- cation Factor	Recovery (%)
Cytosolic extract	30,000	1,020,000	34	1	100
Ion Exchange (Pool)	991	459,000	463	14	56
Gel Filtration <u>Filtration</u>	18.4	100,980	5,600	165	22

Please replace the paragraph on page 96, lines 18-29, with the following:

Purification of the dsRNase activities from liver ~~nuclei~~ nuclei and cytosol suggests that at least two dsRNases with differing properties are capable of cleaving double-strand RNA. The nuclear dsRNase eluted at higher NaCl concentrations from the ion exchange

APPLICANTS: Stanley T. Crooke
SERIAL NO: 09/479,783

DOCKET NO: ISIS-4313

column than the cytosolic dsRNase. However, both require Mg^{++} and cleave at several sites within the oligoribonucleotide gap. Both require a duplex substrate and can cleave oligoribonucleotides in a duplex that is made up of oligoribonucleotide "sense" and a 2' methoxy phosphorothioate chimeric "antisense" strand when the duplex has phosphorothioate or phosphorothioate-2' methoxy nucleoside wings.